SUMMARY AND CONCLUSION

The importance of natural products for medicine and health has been tremendous throughout the evolution and people are always in association with plants for his food, shelter, medicine etc. The use of natural ingredients in drugs, cosmetics and food industries are increasing and these include extracts from various medicinal and aromatic plants. The current growing trend of dependence on medicinal plants is due to several reasons, including escalating faith in traditional systems of medicines, natural and herbal remedies and also as promoting a habit of going back to nature. Kerala, with its rich diversity and age old practice of Ayurveda can significantly contribute to traditional systems of medicine to combat many diseases. The development of new types of diseases and evolution of new drug resistant strains of pathogens forced the chemists and biologists to search for new drug molecules among the natural resources. This thesis involves the study of three medicinal plants belonging to three different important families viz, *Cyperus rotundus* (Cyperaceae), *Stereospermum colais* (Bignoniaceae) as well as the well known medicinal plant *Zingiber officinale* (Zingiberaceae) as the third.

The first chapter gives an overview of biologically active natural products with special reference to antioxidant, antidiabetic, antiinflammatory and antimicrobial molecules from terrestrial sources. The overwhelming effect of free radicals and reactive oxygen species (ROS) in degenerative diseases and conditions like atherosclerosis,
cancer, ageing etc. and the importance of naturally occurring antioxidants for the control of such free radicals are also portrayed in the first chapter.

Chapter 2 of the thesis deals with the isolation of phytochemical constituents of the medicinal plant *Cyperus rotundus* and its antioxidant and radical scavenging potential. Here a brief outline of the genus *Cyperus* is given along with detailed survey of literature on the phytochemistry and biological potential of *C. rotundus*. Three sesquiterpenoids with identical molecular weight (solavetivone (I), aristolone (II) and nootkatone (III)) and two flavonoids (quercetin (IV) and amentoflavone (V)) have been isolated from the acetone extract. Solavetivone has been reported for the first time from the species.
HPTLC and reverse phase HPLC methods were used for the quantification of sesquiterpenoids isolated from the acetone extract of the rhizomes of *C. rotundus*. The chromatographic methods were validated in terms of their linearity, correlation coefficient, precision, accuracy, LOD, LOQ etc. Among the sesquiterpenoids isolated, nootkatone present in highest amount (0.5%) in plant material. The components showed good linearity with a correlation coefficient value of $r = 0.98$ in a relatively wide concentration range. The evaluation of LOD and LOQ suggested the sensitivity of the method. Screening of total antioxidant assay by phosphomolybdenum reagent and DPPH radical scavenging potential of solavetivone, aristolone and nootkatone showed good activity and nootkatone possessed higher antioxidant and radical scavenging potential compared to standard ascorbic acid and gallic acid respectively. The studies revealed the presence of biologically active sesquiterpenoids in the rhizomes of *C. rotundus*.

Chapter 3 of the thesis describes the studies on the roots of *Stereospermum colais*, a Bignoniaceae plant belonging to the genus *Stereospermum* which is used extensively in
Ayurveda. Here a brief outline of the genus *Stereospermum* is portrayed along with a literature survey of *S. colais*. The antioxidant activity studies on the acetone (ASC) and methanol (MSC) extracts of *S. colais* was analyzed by various *in vitro* models. The extracts were effective in scavenging certain free radicals and reactive oxygen species. Acetone extract showed promising xanthine oxidase inhibition potential compared to standard allopurinol. The lipid peroxidative inhibition potential was also shown by acetone extract comparable to the standard BHT and found to decline with time while methanol extract showed moderate activity. In thermal oxidation conditions also, acetone extract effectively inhibit the lipid oxidation of sunflower oil. Acetone extract also showed good radical scavenging properties in DPPH radical scavenging assay and superoxide radical scavenging assay. It showed nitric oxide scavenging activity and good reducing power. Since acetone extract possess high antioxidant potential, column chromatographic method was done to isolate the active components. Column chromatographic fractionation of acetone extract using varying polarities of hexane-ethyl acetate solvent system yielded 7 compounds viz, β-sitosterol (VI), 2-(4′-hydroxyphenyl) ethyl undecanoate (VII), 2-(4′-hydroxyphenyl) ethyl pentadecanoate (VIII), 5α-ergostan-7, 22-dien-3β-ol (IX), ursolic acid (X), lapachol (XI) and pinoresinol (XII). All the compounds were identified for the first time from the species.
The compounds were screened for its DPPH radical scavenging potential and pinoresinol showed the good antioxidant potential compared to standard gallic acid. Ursolic acid and lapachol also showed activity moderate to standard compound and other compounds failed to show the activity. This study portrayed the presence of antioxidant molecules in *S. colais*. Ursolic acid, lapachol and pinoresinol were also screened for the inhibition of α-glucosidase and α-amylase enzymes. The results showed that the compounds posses significant inhibition potential against enzymes. Inhibition of
advanced glycated end products also showed significant potency of the molecules against diabetes and other metabolic disorders. The order of activity based on these assays is ursolic acid>lapachol>pinoresinol.

Results of the *in vitro* protein denaturation inhibition potential showed that 5α-ergostan-7, 22-dien-3β-ol, ASC and MSC substantially inhibited protein denaturation. 5α-ergostan-7, 22-dien-3β-ol showed promising activity compared to standard diclofenac sodium even at low concentrations. During the *in vivo* assay to check antiinflammatory effect, the isolated compound 5α-ergostan-7, 22-dien-3β-ol as well as the acetone and methanol extracts of *S. colais* significantly inhibited carrageenan induced paw edema in rats with 83%, 33% and 50% inhibition respectively at 5th hour. Evaluation of antibacterial and antifungal effects of ursolic acid and lapachol showed a significant clearance zone by ursolic acid against *Klebsiella pneumonia* and *Trichophyton rubrum*.

Chapter 4 describes the biological potential of rhizomes of *Zingiber officinale*. A detailed description of the biological potential of *Z. officinale* is given in the introduction part of chapter 4. The rhizomes were sequentially extracted with solvents like hexane, ethyl acetate, methanol, 70% methanol-water and water. The antioxidant potential of sequential extracts estimated using various *in vitro* models viz., total phenolic content, DPPH radical scavenging activity, superoxide radical scavenging activity and total reducing power are also discussed in detail in chapter 2.

The results showed that ethyl acetate extract of ginger (EAG) possessed antioxidant activity as is evident from the results of various *in vitro* assays compared to other extracts. DPPH radical scavenging activity showed that ethyl acetate extract of ginger possessed highest activity compared to standard gallic acid. EAG exhibited
comparably higher super oxide scavenging potential than catechin, which served as positive control. The extract possessed good reducing power and contains phenolic constituents. Thus it could be summarized from the results of various assays in chapter 4 that ethyl acetate extract of the rhizomes of *Z. officinale* possessed potent biologically active molecules. Owing to the presence of more phenolic constituents in ethyl acetate extract, detailed biological studies including hypoglycaemic effect, inhibition on adipocyte differentiation etc. was done for EAG only. Inhibition of $\alpha$-glucosidase and $\alpha$-amylase enzymes and glycated end products were also carried out for EAG and showed potent activity. Inhibition of low density lipoprotein oxidation, angiotensin converting enzyme activity, cyclooxygenase (COX) enzyme etc. was carried out and EAG showed promising results compared to standards.

Cytotoxicity of EAG was checked against L6, C2C12 and 3T3L1 cell lines within 10-100 $\mu$g/ml concentration. From the results, it was confirmed that EAG was safe to selected cell lines within 50 $\mu$g/ml when incubated for a time period of 24 hr. EAG was able to ameliorate $H_2O_2$ induced oxidative stress in C2C12 cell lines at a concentration of 100 $\mu$g/ml when administered for 1 hr prior to stress induction. The change in ROS production in cells was evaluated by DCFDA fluorescence and measured using flow cytometer.

A reverse phase HPLC method was employed to find out the active pungent constituents, gingerols and shoagols present in the successive extracts of ginger. Ethyl acetate extract of ginger possessed highest amount of pungent constituents and 6-gingerol was found to be the major constituent resolved at a retention time of 3.06 as it is visible from the chromatogram, which is responsible for the biological potential of the extract.
Owing to the insoluble nature of 70% methanol-water and water extracts, quantification was not done in these extracts. Hence the use of ginger appears to be safe and its effect seems to be mighty and amazing in most of the biological studies.

In conclusion, medicinal plants *Cyperus rotundus* and *Stereospermum colais* have been analysed for their phytochemical constituents. Also, the positive results obtained from biological activity studies such as antioxidant, antiinflammatory and antimicrobial activity on the isolated compounds/extracts add on to the medicinal properties of these plants. Apart from that, ethyl acetate extract of *Zingiber officinale* (ginger) rhizomes has been shown to have very good biological potential including glucose lowering and adipocyte differentiation inhibitory effect.